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FABRICATION OF CURCUMIN LOADED NANO POLYCAPROLACTONE/CHITOSAN NONWOVEN FABRIC VIA ELECTROSPINNING TECHNIQUE

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ABSTRACT

Cucurmin loaded poly ε–caprolactone/chitosan (PCL/CTS) nanoscale nonwoven fabric was successfully fabricated using electrospinning, a facial and efficient method. The surface tensions of PCL/CTS blend solutions in various ratios were measured to evaluate the influence of PCL/CTS ratio on fibers formation. The effects of process parameter such as the applied voltage, tip–collector distance and the solution flow rate on the fiber generation and morphology of final fiber were optimized. Nanofibers morphology and structure were characterized by scanning electron microscopy (SEM) and transmission electron microscopy (TEM), respectively. The prepared fibers had a smooth surface and fine morphology. The diameter of fibers ranges from 200 nm to 400 nm. The release kinetics of curcumin loaded samples were also analyzed via *in vitro* technique. Results demonstrated that the polycaprolactone/chitosan–based nanofibers encapsulate curcumin is a potential material for wound healing acceleration.

Keywords: poly ε–caprolactone, chitosan, electrospinning, wound healing.

1. INTRODUCTION

Electrospinning is a technique utilizing electrostatic force to produce polymer fibers with diameters ranging from nanometers to several micrometers using the polymer solution. This is the most advanced methods in manufacturing high–performance nanofibers. These have been introduced into various technological fields because of their distinct specifications, such as high aspect ratio, porosity, and special chemical and physical properties which result from their unique structure [1]. Based on such characteristics, nanofibers have been applied for many medical applications such as drug delivery system [2, 3], tissue engineering [4, 5], and wound healing [6, 7]. In particular, to obtain the desirable size and morphology of fibers which will

suitable for medical application, the optimum parameters of the process of electrospinning are required.

Chitosan (CTS), a natural polysaccharide derived from chitin, shows advantageous characteristics such as biocompatibility, biodegradability, hydrophilicity, non–toxicity and antimicrobial activity. Therefore, chitosan will be a promising material for biomedical use [8, 9] However, numerous studies report that the difficulties encountered when electrospinning pure chitosan [10, 11]. Moreover, the weakness of mechanical properties will limit the practical performance of Chitosan in the medical application [12].

Blending chitosan with a synthetic polymer may provide a superior material that combines the benefits of both, showing a good tissue compatibility and improved mechanical properties. Poly ε–caprolactone (PCL) is a candidate for the synthetic polymer to be mixed with chitosan because of its biocompatibility, biodegradability, non–toxicity and good mechanical properties [13]. PCL is also studied for biomedical applications but suffers from hydrophobicity and lack of cell–recognition sites for the support of cell adhesion [14], both of which can be supplied by chitosan. Moreover, it has been stated that PCL shows a good miscibility with various polymers and improves the process ability of some polymers [15]. Hence, blending PCL with chitosan would most likely assist the electrospinning process of chitosan [16].

In this work, curcumin loaded chitosan/PCL nanofilm was produced by electrospinning technique. A parametric study was conducted to determine the effect of several parameters namely PCL/CTS ratio (PCL/CTS), applied voltage (U), flow rate (Q) and electrospinning distance (L) which enable successful fiber production. A correlation between different product morphologies and processing parameters was also established. The suitability for wound healing of PCL/CTS nanoscale nonwoven fabric was evaluated by testing its drug release *in vitro*.

2. MATERIALS AND METHODS

2.1. Materials

Curcumin was bought from National Institute of Medicinal Materials. PCL (MW = 70,000 – 90,000) was supplied by Sigma–Aldrich. Chitosan (degree of deacetylation 80–85%, MW: 100,000 – 300,000) was supplied by Acros Organics. Phosphate buffered saline (PBS) used for in vitro release study was bought from Sigma–Aldrich. All solvents (formic acid and acetone) were purchased from Samchun (Korea).

2.2. Methods

2.2.1. Electrospinning PCL/CTS nonwoven fabric

PCL were dissolved in acetic acid/acetone (3:1 w/w) and stirred at room temperature for 30 min. Chitosan was added to PCL solutions to achieve the ratio PCL/CTS: 9/1, 8/2, 7/3, 6/4, and 5/5. The polymer concentration of all solutions were 10 wt%. All the polymer solutions were stirred for 2 h. The PCL/CTS solution was stored in a 20 mL syringe and set up in a pump. Collector was covered by an aluminum foil in order to retrieve electrospun fibers. The experiment was conducted at room temperature, in ambient air which has moisture is around 80%. The samples were dried at room temperature for 48 h to completely remove the remaining formic acid and acetone after electrospinning process. The optimum process parameters obtained from this section were used to fabricate curcumin loaded PCL/CTS fabric.

2.2.2. Electrospinning curcumin loaded PCL/CTS nonwoven fabric

With a set of parameters above, curcumin loaded PCL/CTS nonwoven fabric fabrication was carried out. The PCL/CTS solutions were prepared as presented above. Curcumin was last added into the solutions with the different amount (1 wt%, 3 wt%, 5 wt%, calculated based on the amount of PCL and chitosan). After that, the solutions were sonicated in 2 h before the electrospinning operation. The electrospun fabrics were dried at room temperature for 48 h in order to completely remove formic acid and acetone.

2.2.3. In vitro drug release

Three marked fibrous mats samples (3×3 cm) were dispersed in 3 vials with 20 mL phosphate buffer solution 1 % Tween 20 at 37 °C with pH value at 7.4. All the supernatants were pipetted out periodically and replaced with an equivalent volume of fresh phosphate buffer solution. These supernatants were then used for determining the amount of released drug.

2.3. Analytical methods

2.3.1. Surface tension analysis

The surface tension of different ratio PCL/CTS solutions was measured using contact angle analyzer (Dataphysics OCA 20, Nation Key Lab for Polymer and Composite Materials).

2.3.2. Morphology analysis

The morphology of fibrous mats was analyzed using scanning electron microscopy (SEM – Hitachi S4800, National Institute of Hygiene and Epidemiology (NIHE)). The average fiber diameter of electrospun samples was determined from SEM images using the Image–J software. The structure of the nanofibers was characterized by transmission electron microscopy (TEM – JEOL JEM 1400, Nation Key Lab for Polymer and Composite Materials).

2.3.3. In vitro drug release

The concentration of released drug was determined by UV–Vis spectrometer at 420 nm (Thermo Fisher Genesys 10S, Faculty of Chemical Engineering – Industrial University of Ho Chi Minh City). The percentage of curcumin released was determined using the equation:

Curcumin release (%) = (curcumin released at time/total curcumin loaded in fibrous mats) ×100

3. RESULTS AND DISCUSSION

3.1. PCL/CTS electrospun nonwoven fabrics

3.1.1. Effect of ratio PCL/CTS

The electrospun fiber morphology is dependent on the surface tension of polymer solutions. Generally, the surface tension of a polymer solution will affect the electrospinning process by changing the cone–jet stability. In electrospinning process, the fiber will be formed when the electric force can overcome the solution surface tension. Furthermore, in the mixture of the polymer solution, the surface tension strongly depends on the concentration of each polymer

component. Thus, PLC/CTS ratio will affect the electrospinnability by changing the solution surface tension. The surface tension of polymer solution prepared from different PCL/CTS ratios were examined.

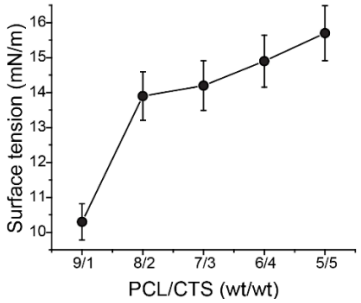

Figure 1. The surface tensions of five solutions with different PCL/CTS ratios.

Figure 1 shows the surface tensions of five solutions with different PCL/CTS ratios: 9/1; 8/2; 7/3; 6/4 and 5/5. It can be seen that the surface tension of polymer solution decreases while reducing the rate of Chitosan. To evaluate the effect of PCL/CTS ratio which changing the surface tension of polymer solution on the size and morphology of fibers, a set of nonwoven fabrics were prepared. PCL/CTS solutions with ratio of 9/1; 8/2; 7/3; 6/4 and 5/5 were electrospinning with the following process parameter: L = 7 cm, Q = 0.3 mL/h, U = 24 kV.

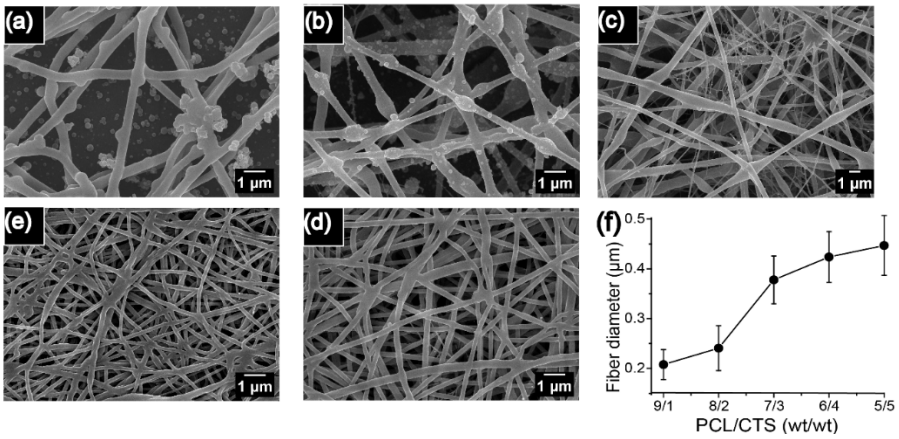

Figure 2. The SEM images of PCL/CTS electrospun fabrics made from different ratio of (a) 5/5, (b) 6/4, (c) 7/3, (d) 8/2, (e) 9/1 using following parameters: L = 7 cm, Q = 0.3 ml/h, U = 24 kV and fiber diameters of these samples (f).

Figure 2 shows the SEM images of five fiber samples fabricated from five solutions with different PCL/CTS ratios: 9/1; 8/2; 7/3; 6/4 and 5/5. Because of high surface tension, the liquid cone–jet was not stable. The surface tension tends to convert the cone–jet into spherical droplets to reduce the surface area, resulting in the formation of the beads on the surface of the fibers fabricated from PCL/CTS solution with PCL/CTS ratio of 5/5 and 6/4, as shown in Figure 2a and Figure 2b. When the surface tension of solution was low enough, the electric field can generate a stable cone–jet which will lead to the formation of continuous fiber without the bead, as presented in Figure 2c, d, e. The average fiber diameter decreases from 209 nm, 242 nm, 379 nm, 425 nm to 448 nm when PLC/CTS ratio decreases from 9/1; 8/2; 7/3; 6/4 and 5/5,

respectively, as given in Figure 2f. The higher surface tension prevents the cone–jet formation and also reduces the evaporation of solvents, elongation and thinning, resulting in the increase of the average diameter of fibers when the concentration of chitosan in solution increases. It can be observed that at PCL/CTS ratio of 9/1, a substantial number of fibers were produced without beads. The fibers formed from this ratio also have good morphology. This PCL/CTS ratio was thus considered the optimum value and was used for evaluating the effect other parameters in this study.

3.1.2. Effect of the high – voltage power supply

In this step, the influence of the high–voltage power supply on the size and morphology of electrospun fibers was investigated. The following process parameters were used to prepare PCL/ CTS nano nonwoven fabrics: PCL/CTS = 9/1, Q = 0.3 mL/h, L = 7 cm, U = 15 kV, 18 kV, 24 kV. Figure 3 exhibits the SEM images of PCL/CTS electrospun fibers prepared using three different high–voltage power. As shown in Figure 3, the fibers tend to aggregate together when increasing the applied voltage. The higher electrostatic force can lead to the formation of multi–jet, resulting in several fibers were generated on the tip of the needle at the same time. These fibers can stick together to form a bigger fiber. This phenomenon can also limit the evaporation of the solvent, which may favor the formation of increased fiber diameter. The average fiber diameter increased from 158, 200, to 209 nm when the applied voltage rose from 15, 18, to 25 kV, respectively. These data suggest that to fabricate thinner fibers, smaller nozzle diameter is desired. The results indicate that more uniform and homogeneous fibers were obtained when utilizing power supply of 15 kV. Hence, this value will be use to investigate the influence of other parameters in this study.

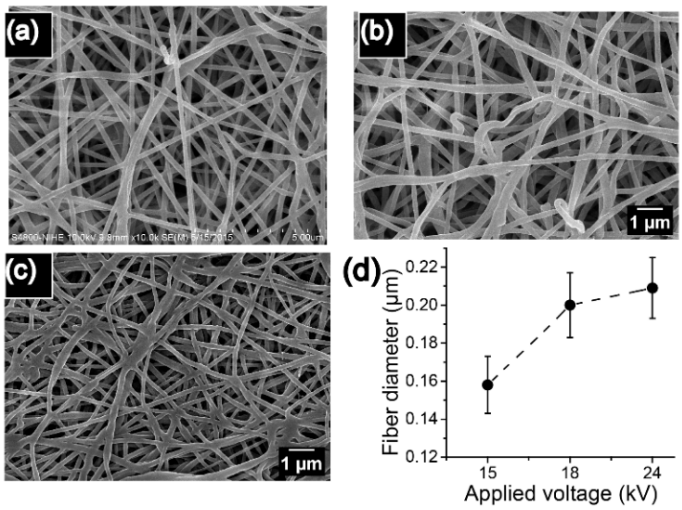

Figure 3. The SEM images of PCL/CTS electrospun fabrics prepared from polymer solution with PCL/CTS ratio of 9/1, using following parameters: Q = 0.3 mL/h, L = 7 cm and different high – voltage supply (a) 15 kV, (b) 18 kV, (c) 24kV and fiber diameters of these samples (d).

3.1.3. Effect of the flow rate

In this step, the influence of flow rate on the size and morphology of PCL/CTS electrospun fibers was investigated. A set of nonwoven fabrics were prepared using these parameters: PCL/CTS = 9/1, U = 15 kV, L = 7 cm, Q = 0.1 mL/h, 0.3 mL/h, 0.5 mL/h. Figure 4 exhibits the

SEM images of PCL/CTS electrospun fibers made from three different flow rates. At the flow rate of 0.1 ml/h, polymer solution ejected from the tip was slowly, so the formed con–jet was unstable, leads to uneven fiber and branches in the final sample. The stable con–jet was gained when the flow rate increased to 0.3 mL/h. At the result, the fibers obtained using Q = 0.3 mL/h have a uniform and smooth morphology. When the flow rate increased to 0.5 mL/h, the polymer solution is ejected from the needle too fast, the pulled out solution polymer made the bigger con–jet, resulting in the bigger and narrower fibers. It can be seen in Figure 4 that the average diameter of fibers increased from 158, 188 to 208 nm when changed flow rate from 0.1, 0.3 to 0.5 mL/h, respectively. The results indicate that the collected fibers had the best morphology when using the flow rate of 0.3 mL/h. Therefore, the flow rate of 0.3 mL/h was used for other experiments in this study.

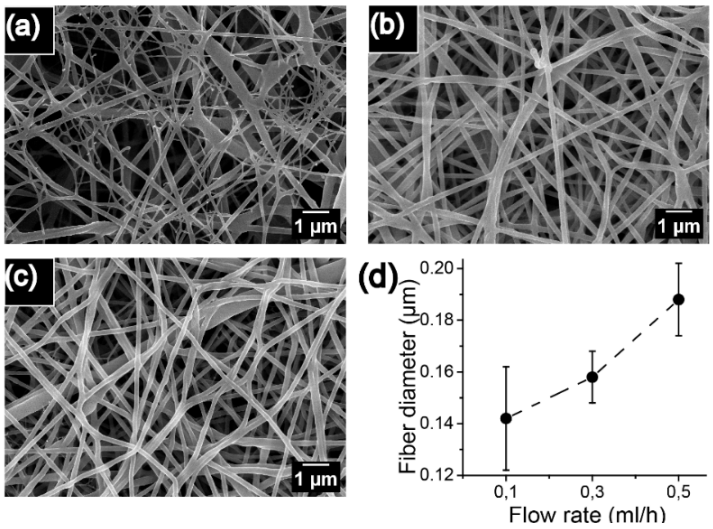

Figure 4. The SEM images of PCL/CTS electrospuns fabrics prepared from solution with PCL/CTS ratio of 9/1, using following parameter U = 15 kV, L = 7 cm with different flow rate (a) 0.1 mL/h, (b) 0.3 mL/h, (c) 0.5 mL/h and fiber diameters of these samples (d).

3.1.4. Effect of electrospinning distance

To evaluate the effect of electrospinning distance on the size and morphology of PCL/CTS electrospun fibers, a set of PCL/CTS fabrics were prepared using these parameters: PCL/CTS = 9/1, U = 15 kV, Q = 0.3 mL/h, L = 4 cm, 5.5 cm, 7 cm and 8 cm. The electrospinning process is closely related to the evaporation rate of the solvent using for dissolving polymers. The tip of needle–collector distance can affect the fiber morphology by changing the flight time of the fiber formed from liquid cone–jet. In electrospinning, there exists a minimum electrospinning distance that allows the sufficient time for most of the solvent to evaporate before arriving at the collectors. If the longer distance is applied, the fiber can have a longer distance to travel, which increase elongation and thinning of fiber, leading to the formation of smaller fiber. The SEM images of the resultant fibers are given in Figure 5. The average fiber diameters were 300, 208, 158 and 145 nm, respectively, when the needle tip–collector distances were 4, 5.5, 7 and 8 cm. It can be seen that electrospun fibers obtained the highest size uniformity and the best morphology when using the electrospinning distance at 8 cm. Thus, 8 cm was considered at the optimum distance for electrospinning operation in this study.

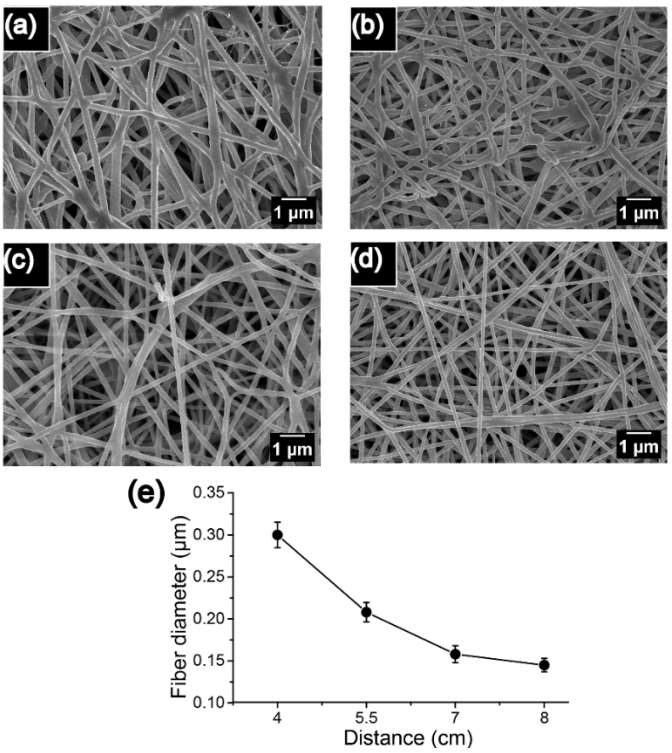

Figure 5. The SEM images of PCL/CTS electrospun fabrics prepared from solution with PCL/CTS ratio of 9/1, using following parameters U = 15 kV, Q = 0.3mL/h with different electrospinning distance (a) 4 cm, (b) 5.5 cm, (c) 7 cm, (d) 8 cm and fiber diameters of these samples (e).

3.1.5. The structure of PCL/CTS nanofiber

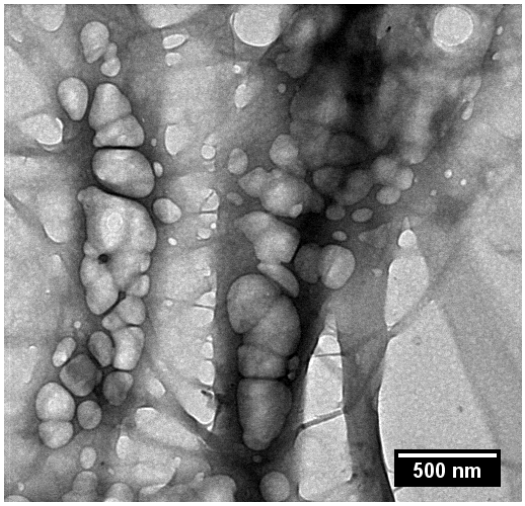

Figure 6. The TEM images of PCL/CTS fiber prepared from solution with PCL/CTS ratio of 9/1, using following parameters U = 15 kV, Q = 0.3ml/h, L = 8cm.

To investigate the blending possibility between PCL and CTS, the structure of the obtained nonwoven fabric produced from solution with PCL/CTS ratio of 9/1 using the optimum

parameters was characterized using TEM method. Figure 6 exhibits TEM image of the fibers. This image reveals that there are many particles dispersion in the fiber. There are two phases in PCL/CTS system: The PCL fiber covers the CTS nanoparticles.

3.2. Curcumin loaded PCL/CTS electrospun nonwoven fabric

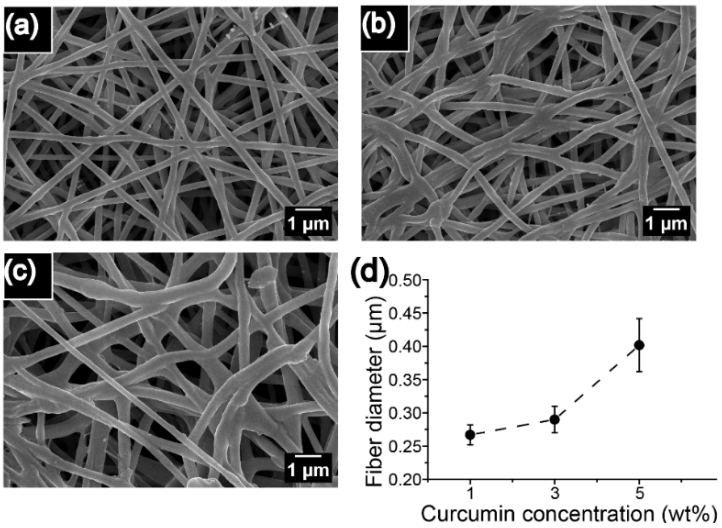

Figure 7. The SEM images of Curcumin loaded PCL/CTS electrospun fabrics prepared from solution with PCL/CTS ratio of 9/1, using following parameters U = 15 kV, Q = 0.3 mL/h, L = 8 cm and fiber diameters of these samples (e).

With the optimum parameters for electrospinning process (PCL/CTS = 9/1, U= 15 kV, L = 8 cm, Q = 0.3 mL/h), curcumin loaded PCL/CTS nonwoven fabrics were fabricated. Figure 7 exhibits the SEM images of curcumin loaded PCL/CTS electrospun fibers made from three different concentrations of curcumin. It can be seen that the diameter of fibers increases when the concentration of curcumin in polymer solutions increases. The higher curcumin concentration leads to the higher viscosity of the spinning solution, which reduced the solvent evaporation rate and prevented the elongation and thinning of electrospun fibers. Resulting in the aggregation of fibers, which can be seen clearly in Figure 7c. When the curcumin concentration increases from 1 wt% to 5 wt%, the average fiber diameter increases from 267 to 402 nm.

3.3. *In vitro* profile

In order to investigate cucurmin release behavior from PCL/CTS nonwoven fabric, three curcumin loaded PCL/CTS samples with different cucurmin concentration were designed, prepared and examined in vitro in PBS (pH = 7.4) at 37 °C. As can be seen in Figure 8, nearly 80% of curcumin was released from all polymeric fabric samples in during the first 100 hours. At the beginning, the concentration difference between inside and outside of the fiber was very large. The drug released very rapidly through the diffusion mechanism. Furthermore, the amount of the drug on the surface and just below the surface fibers also enhances rapid drug release. After 100 h, the drug release from PCL/CTS fibers began slower, and nearly 90 % of total cucurmin released from all samples after 650 h of testing. The release of cucurmin at this time was attributed to the biodegradation of PCL and CTS, which allow the release of cucurmin

inside of the fibers. The drug release speed of PCL/CTS fabric containing 1% was slower than that in PCL/CTS fabric containing 3 wt% and 5 wt% Curcumin. The increase of cucurmin leads to the increase of the amount of drug on the surface of fibers, which rose the speed of drug release in the cucurmin loaded PCL/CT system.

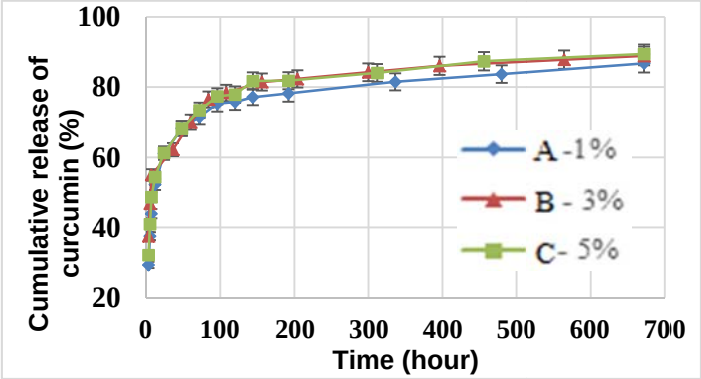

Figure 8. The in vitro release profile of Curcumin loaded PCL/CTS.

4. CONCLUSIONS

The curcumin–loaded PCL/CTS nanofibers were successfully fabricated via electrospinning method to be used for testing curcumin release in vitro. The optimum parameters for electrospinning operation are: PCL/CTS = 9/1, U= 15 kV, L = 8 cm, Q = 0.3 mL/h. The fibers fabricated using these parameters have good morphology with the average diameter from 267 to 402 nm. The drug release behavior of curcumin–loaded PCL/CTS nonwoven fabric was successfully tested, which shows that the drug was released nearly 80% during the first 100 hours. This is the initial review on the mechanism of drug release and influencing factors of the fiber diameter on the drug release from the electrospun fiber in laboratory conditions. The results indicate the ability to reduce the healing time of injury and could replace recent wound dressings in the future.

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